Sensory feedback signal derivation from afferent neurons

Contract No.: **NIH-NINDS-NO1-NS-3-2380**

QUARTERLY PROGRESS REPORT #11

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Principal Investigator: J.A. Hoffer, PhD

Co-investigators: K. Strange, BASc
Y. Chen, PhD
P. Christensen, BASc
D. Crouch, BSc (Hon)
K. Kallesøe, MScEE
C. Kendall, RVT

Origin: School of Kinesiology
Faculty of Applied Sciences
Simon Fraser University
Burnaby, British Columbia V5A 1S6, Canada

Subcontractor: D. Popovic, PhD
University of Miami, Miami, Florida, USA

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I. Summary of the Overall Project

In this study we are exploring the feasibility of extracting 1) cutaneous sensory information about fingertip contact and slip, and 2) proprioceptive sensory information about wrist or finger position. We use implanted nerve cuff electrodes to record peripheral nerve activity in animal models.

Our overall **objectives** for the 3-year duration of this contract are as follows:

1. Investigate, in cadaver material, implantation sites for nerve cuff electrodes from which cutaneous and proprioceptive information relevant to the human fingers, hand and forearm could be recorded.
2. Select a suitable animal preparation in which human nerve dimensions and electrode placement sites can be modeled and tested, with eventual human prosthetic applications in mind.
3. Fabricate nerve cuff electrodes suitable for these purposes, and subcontract the fabrication of nerve cuff electrodes of an alternate design.
4. Investigate the extraction of information about contact and slip from chronically recorded nerve activity using these animal models and electrodes. Specifically,
   a. Devise recording, processing and detection methods to detect contact and slip from recorded neural activity in a restrained animal;
   b. Modify these methods as needed to function in an unrestrained animal and in the presence of functional electrical stimulation (FES);
   c. Record activity for at least 6 months and track changes in neural responses over this time.
5. Supply material for histopathological examination from cuffed nerves and contralateral controls, from chronically implanted animals.
6. Investigate the possibility of extracting information about muscle force and limb position from chronically recorded neural activity.
7. Cooperate with other investigators of the Neural Prosthesis Program by collaboration and sharing of experimental findings.

II. Summary of Progress in the Eleventh Quarter

During the eleventh quarter, we made significant progress obtaining usable neural signals from nerves to individual muscles using small scale tripolar nerve cuffs. We also made significant progress in our histological analysis and initial results are presented in this report. In addition, our collaborative project of applying machine learning techniques to analyzing neural signals and predicting muscle activity has produced outstanding results. Three full manuscripts on the subjects of nerve signal stability, cutaneous nerve signals recorded during locomotion, and machine learning techniques applied to closed-loop control of functional electrical stimulation have been developed.

III. Details of Progress in the Eleventh Quarter

A. Chronic Recordings from Individual Nerves to Forelimb Muscles

Small nerves supplying individual cat forelimb muscles were targeted for instrumentation with cuff electrodes to obtain electroneurographic (ENG) recordings of motor and proprioceptive sensory information during voluntary tasks.

1. Implant Protocol

Nerves to individual muscles in the forelimb were assessed in terms of functional role, accessiblity and ease of instrumentation. Superficial forelimb muscles such as Palmaris Longus (PalL), Flexor Carpi Ulnaris (FCU), Extensor Carpi Ulnaris (ECU), Extensor Digitorum Communis (EDC), and Extensor Digitorum Lateralis (EDL), are usually innervated by small nerves (approximately 0.3 to 1.0 mm in diameter) which branch from the parent nerves (Median, Ulnar, and Deep Radial) below the elbow. These muscle nerves are difficult to instrument due to their fragile nature and the fact that they are usually surrounded by muscles, making it difficult to stabilize the device with respect to the other structures. Furthermore, accessing superficial muscle nerves is often difficult because the nerve entry point is on the underside of the muscle when looking at the surgical site.

Nerves to deep forelimb muscles such as Abductor Pollicis Longus (APL) and Flexor Digitorum Profundus (FDP, 5th head) are similar in diameter to the superficial muscle nerves, but the deep muscle nerves are generally shorter. A significant advantage to targeting deep muscles is that their nerves are more easily accessible from a top view of the surgical site when the superficial muscles are reflected.

We developed a miniature nerve cuff based on the tripolar design reported in Strange et al. (1995), consisting of a small cuff (ID of 0.5 - 1.0 mm, length of 0.5 to 1.0) mounted on a bipolar electromyogram (EMG) patch electrode (Hoffer, 1990). The nerve cuff was closed by an external flap and a locking mechanism involving closing tubes and a baton that has recently been allowed a U.S. Patent (Kallesøe et al, 1994). The patch was sewn to the muscle belly with 8-0 sutures just proximal to the nerve entry point, and the nerve was allowed to drop into the inside of the cuff when it was held open. The outside electrodes of the cuff were shorted together at the cuff and the four lead wires (two cuff - 631 Cooner wire - and two EMG - 634 Cooner wire) exited the device and were routed distal to the device. A strain relief loop was introduced distal to the cuff and the wires were passed subcutaneously to the backpack (Hoffer, 1990; Strange et al., 1995).

In subject NIH 15 we instrumented the nerves to EDL and FDP (5th head) with nerve cuffs of this design, and we instrumented the Median and Radial nerves with proximal and distal nerve cuffs and electroneurogram (ENG) patch electrodes as described in Strange et al. (1995b) and Strange and Hoffer (1995b). The EDL nerve was instrumented with a 5 mm long, 0.6 mm ID cuff mounted on a 5x5 mm patch, and the FDP was instrumented with a 5 mm long, 0.8 mm ID cuff mounted on a 5x5 mm patch. We also implanted EMG patch electrodes on PalL and EDC to record muscle activity during voluntary tasks from a total of four muscles (PalL, FDP, EDC, and EDL).

2. Recordings Under Anesthesia

We periodically monitored the status of the implanted devices by following the compound action potentials (CAPs) as outlined in Strange et al. (1995a,b). The CAP amplitudes recorded from nerves to FDP and EDL declined to 7% and 31% of the day 0 amplitude respectively (on day 14), but showed substantial recovery to 23% and 78% respectively (on day 40). Neural and EMG recordings

from the instrumented muscles during walking on the treadmill showed increasing amplitudes and confirmed the recovery in the instrumented nerves, which was similar to earlier observations from cutaneous nerves (Strange et al., 1995a,b).

While the subject was under anesthesia, we investigated the ENG signals in nerves to FDP and EDL during manual oscillations of the paw and digits. All physiological signals were processed according to the methods of Strange and Hoffer (1995). In one experiment the paw was held at zero degrees flexion, and the digits were rigorously flexed and extended at a rate of 2 to 3 cycles per second. Data from this experiment are presented in Figs. 1 and 2 which show neural signals in the top four traces (FDP, EDL, Rad, and Med) and EMG signals in the bottom four traces (PalL, FDP, EDC, and EDL). All signal traces are displayed in microvolts.

We observed that all four nerve signals were modulated with the manual oscillations of the digits, while none of the EMG signals showed significant activity or modulation. These observations demonstrated that reflexes were almost totally suppressed by anesthesia and that recorded neural signals were predominately sensory in nature. The cutaneous Radial and Median signals showed bursts of activity with each perturbation regardless of direction (flexion or extension), while the FDP nerve showed a burst only during (or after) digit extension and the EDL showed a burst only during (or after) digit flexion. The direction of the perturbation suggested that the recorded signals were produced by spindle afferents that fired as the passive muscle was stretched. Figure 2 shows the same data as Fig. 1 on an expanded time scale. The opposite directional dependence of the FDP and EDL ENG signals is clearly demonstrated.

Further experiments under anesthesia showed that the FDP and EDL nerve signals were sensitive to both position and velocity of the perturbation. The manual oscillation of the paw and digits was difficult to calibrate and repeat reliably, and an improved perturbation task will be required to fully characterize the responses in the nerves to individual muscles when the subject is under anesthesia.

3. Recordings During Walking on the Treadmill

We examined the FDP and EDL signals recorded during walking on the treadmill to assess the information available from nerves to individual forelimb muscles. The recording protocol, a discussion of signals recorded from Median and radial cutaneous nerves during walking, and a discussion of recorded EMG signals and the role of selected forelimb muscles are found in Strange and Hoffer (1995).

Figure 3 presents data recorded from NIH 15 on day 28 post implant. The treadmill was level and the speed was 0.5 m/s. Once again, the top four traces represent neural signals (FDP, EDL, Rad, and Med) and the bottom four represent EMG signals (PalL, FDP, EDC, and EDL). All signals are displayed in microvolts. The first observation to note is that all four muscle signals are modulated with the step cycle as shown by the lines signifying contact and lift-off. The PalL showed the highest amplitude signals and was activated just prior to contact and during most of the stance phase. The FDP had relatively low levels of EMG activity throughout the step cycle, possibly as a result of partial nerve injury due to instrumentation, but it was well modulated with activity occurring prior to lift-off and into the swing phase. The EDC and EDL were both modulated with the step cycle, with peaks in activity occurring just prior to contact to stabilize the wrist joint. The timing and relative amplitudes of muscle activity generally agreed with earlier recordings as reported in Strange and Hoffer (1995).

The nerve signals shown in Fig. 3 are all modulated with the step cycle, with the Radial and Median nerve signals similar to recordings in earlier experiments (Strange and Hoffer, 1995). The FDP is strongly modulated with the step cycle with higher levels of activity during stance and peaks occurring prior to lift-off. The signal was surprisingly strongly modulated with the step cycle, considering the partial injury to the nerve as assessed by the CAP. The nerve to each instrumented muscle contained both motor and sensory fibres, and their relative contribution in whole nerve

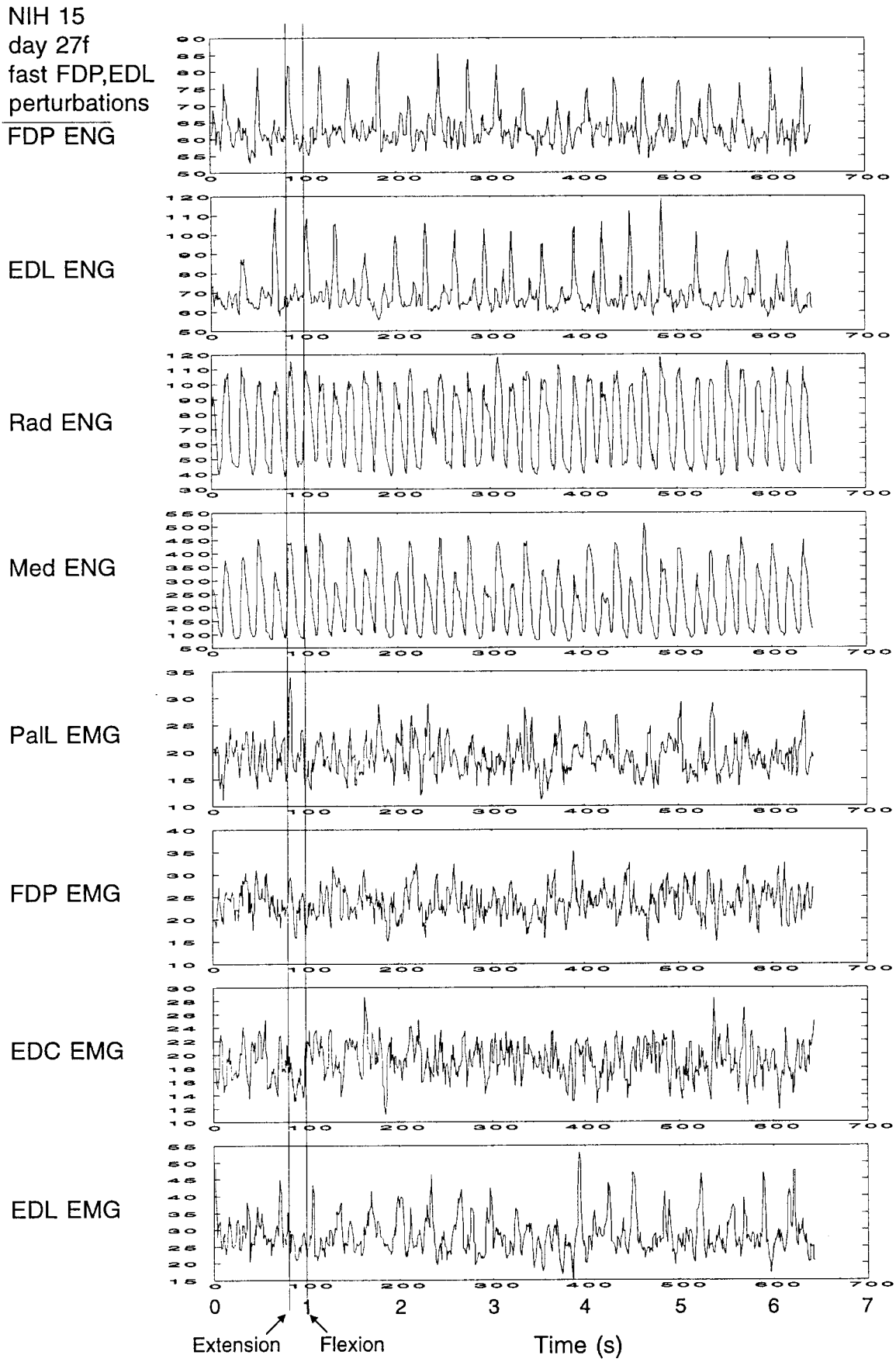

Figure 1: ENG and EMG data recorded under anesthesia from cat forelimb, with mechanical perturbations of the wrist and digits (data from NIH 15)

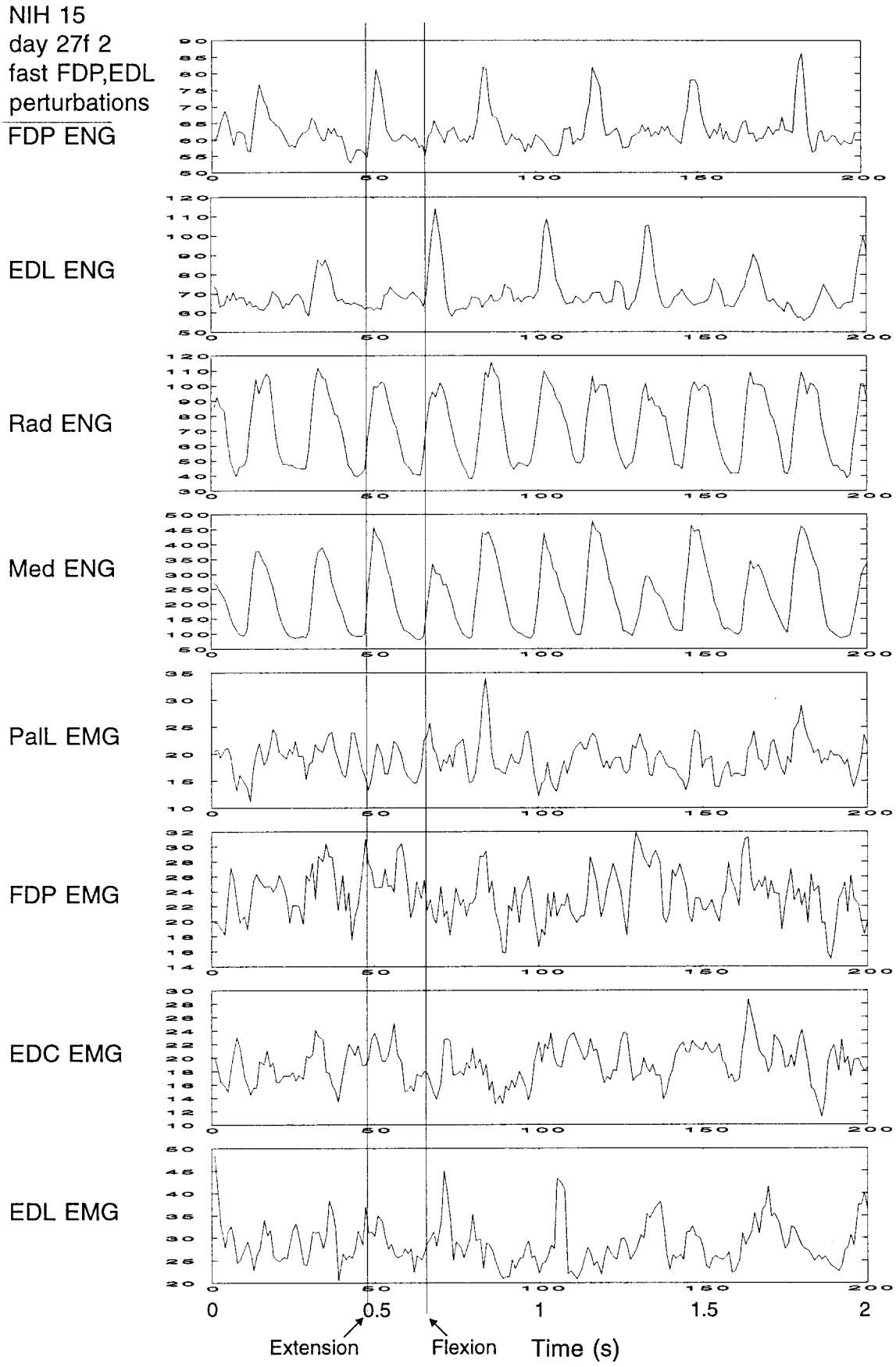

Figure 2: ENG and EMG data recorded under anesthesia from cat forelimb, with mechanical perturbations of the wrist and digits (data from NIH 15)

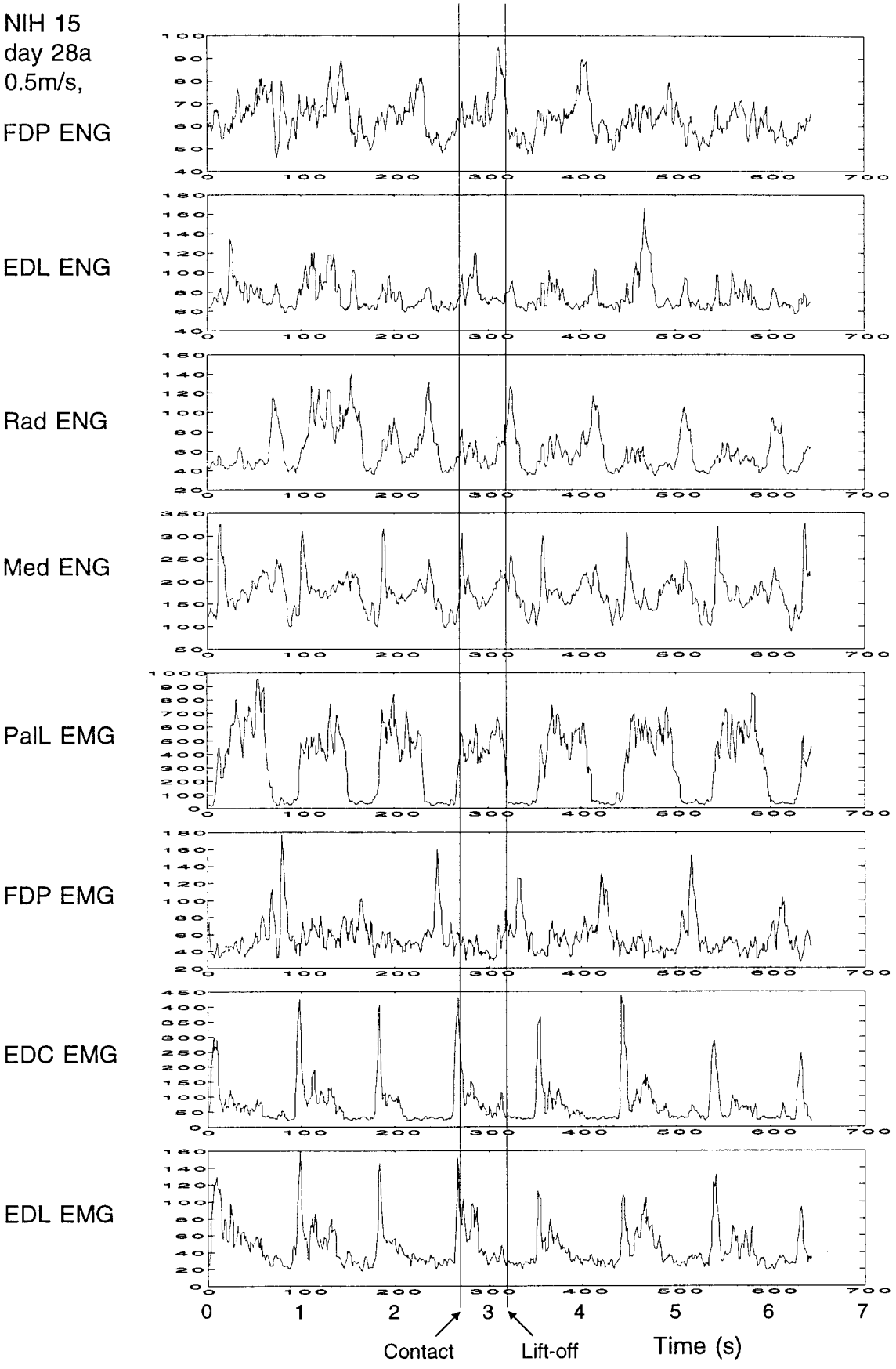

Figure 3: ENG and EMG data recorded from cat forelimb during walking on a treadmill (data from NIH 15, level treadmill, 0.5 m/s)

signals shown in Fig. 3 is unknown. The FDP ENG showed increasing activity throughout stance, possibly a sensory signal resulting from loading the muscle as the body moved forward and the muscle lengthened. The large burst in ENG prior to lift-off is thought to be a volley of motor signals activating the muscle to provide force during lift-off and swing, and the sharp decrease in activity following lift-off indicated absent motor signals and absent sensory signals as the muscle is inactive and unloaded.

The EDL showed modulations with the step cycle, although the signal is quite variable and the relationship between EDL ENG and EMG is not as clear as in the FDP recordings. The limited, variable activity of the extensor muscles during walking may play a role in the variability in the EDL ENG recordings shown in Fig. 3 and represents an interesting aspect of control that warrants further investigation.

4. EMG Contamination of Nerve Cuff Signals

We investigated the amount of EMG contamination of nerve cuff signals recorded in NIH 15 during walking, and determined the EMG rejection properties of the implanted nerve cuffs and the ENG patch electrode. ENG signals were sampled at 20 kHz and processed by the method described in Strange and Hoffer (1995). FFTs of the signals produced the power spectra of the four ENG signals both before and after secondary Ithaco filtering.

Figures 4 and 5 present the power spectra of the four ENG signals recorded in NIH 15 walking on a level treadmill on day 28, which is the same recording session as shown in Fig. 3. Figure 4 shows the spectra for FDP, EDL, Radial, and Median ENG signals (top to bottom), along with device dimensions, filtering characteristics, and calculated ENG-to-EMG signal-to-noise ratio (SNR) for each signal. The SNR was calculated by integrating the spectra up to 1 kHz (the frequency range for EMG), and integrating the spectra from 1 kHz to 10 kHz (the frequency range for ENG). The spectra are derived from signals in volts and the units for the spectra are volts squared.

All four panels in Fig. 4 show that considerable EMG pickup is present in the recorded signal following bandpass filtering with the Bak amplifiers. The peaks of EMG occur at 300 to 600 Hz and are approximately two orders of magnitude larger than the ENG component above 1 kHz, seen in Fig. 4 as the bumps on the falling edges of the EMG peaks.

Figure 5 presents the signals from Fig. 4 following secondary highpass filtering at 1 kHz with Ithaco filters (Strange and Hoffer, 1995). The EMG component has been reduced and the peaks in the signals now occur at or above 1 kHz. The SNR for each device is shown in the figure (average = 4.65 $\pm$2.9, n = 4), along with the increase in SNR due to Ithaco filtering. These values are comparable to SNR values reported for Ulnar, Median, and Radial recordings in Strange and Hoffer (1995). The EDL and Radial ENG signals appear to still contain some EMG components as shown by the sharp peak at 1 kHz, although the majority of EMG contamination has been filtered out.

The data in Figs. 4 and 5 suggest that highpass filtering of signals from nerve cuff and patch electrodes can remove the majority of EMG contamination and produce clean ENG signals as shown in Fig. 3. Nerve cuff signals essentially free of EMG contamination may be used as feedback signals for closed-loop control of functional electrical stimulation systems. In particular, feedback signals from individual nerves to forelimb muscles would be useful for controlling limb position during reaching or other voluntary activities.

NIH 15, day 28a
0.5 m/s, level, fsamp = 20 kS/s
file: psd28a2.idw

FM7, FDP nerve cuff
0.8 mm ID, 5 mm long
BP filtered @ 50Hz-1kHz, Bak
ENG:EMG ratio = 0.37
(threshold at 1 kHz)

FM8, EDL nerve cuff
0.6 mm ID, 5 mm long
BP filtered @ 50Hz-1kHz, Bak
ENG:EMG ratio = 0.41
(threshold at 1 kHz)

FM9, Radial nerve patch
10 * 30 mm
BP filtered @ 50Hz-1kHz, Bak
ENG:EMG ratio = 0.068
(threshold at 1 kHz)

FM10, Median nerve cuff
2.0 mm ID, 20 mm long
BP filtered @ 50Hz-1kHz, Bak
ENG:EMG ratio = 0.61
(threshold at 1 kHz)

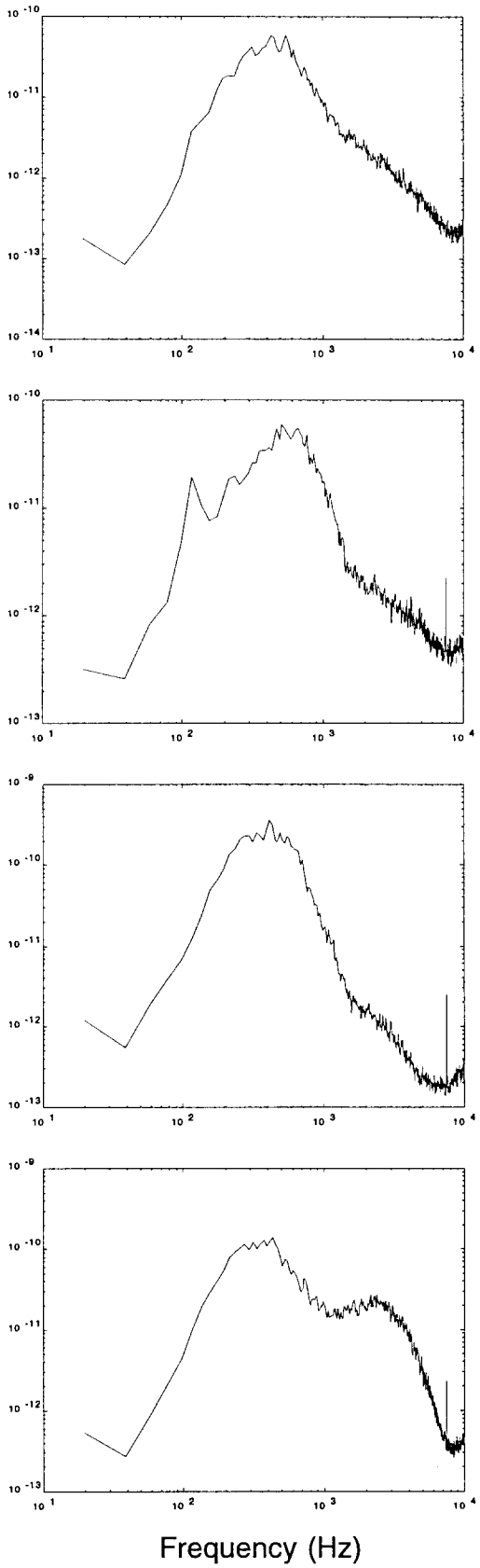

Figure 4: Power spectral densities of nerve cuff recordings from the cat forelimb during walking, prior to high pass filtering with Ithaco filters (data from NIH 15, level treadmill, 0.5 m/s)

NIH 15, day 28a
0.5 m/s, level, fsamp = 20 kS/s
file: psd28a.idw

FM1, FDP nerve cuff
0.8 mm ID, 5 mm long
HP filtered @ 1kHz, Ithaco
ENG:EMG ratio = 8.39 (x22.7)
(threshold at 1 kHz)

FM2, EDL nerve cuff
0.6 mm ID, 5 mm long
HP filtered @ 1kHz, Ithaco
ENG:EMG ratio = 5.47 (x13.4)
(threshold at 1 kHz)

FM3, Radial nerve patch
10 * 30 mm
HP filtered @ 1kHz, Ithaco
ENG:EMG ratio = 2.29 (x33.8)
(threshold at 1 kHz)

FM4, Median nerve cuff
2.0 mm ID, 20 mm long
HP filtered @ 1kHz, Ithaco
ENG:EMG ratio = 2.43 (x4.0)
(threshold at 1 kHz)

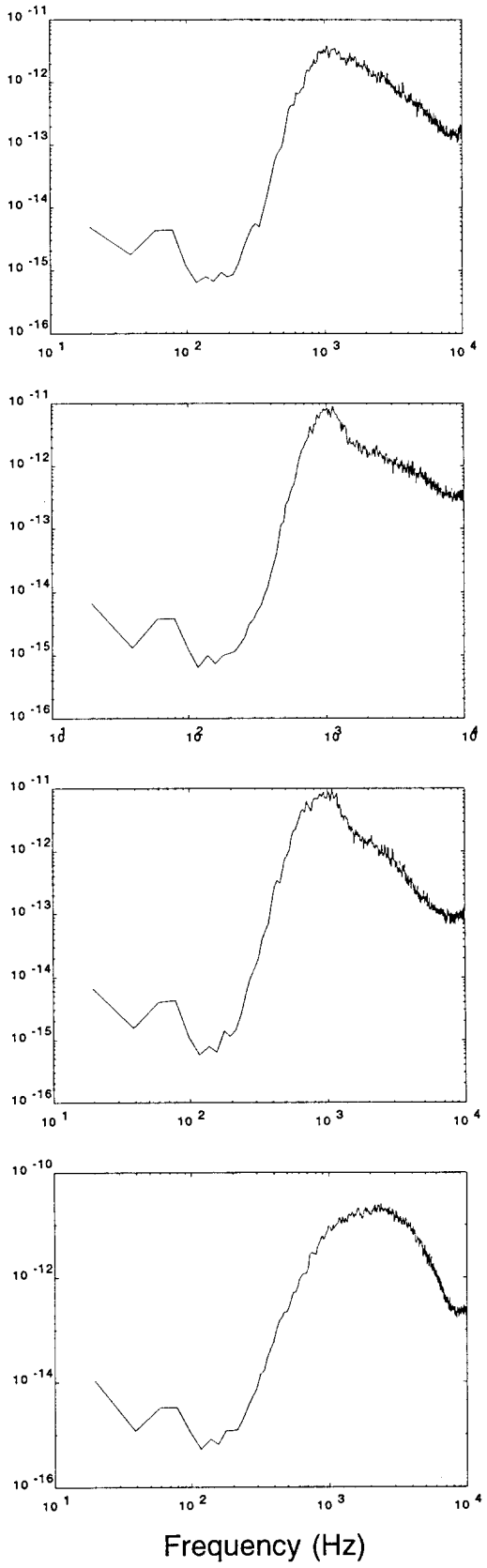

Figure 5: Power spectral densities of nerve cuff recordings from the cat forelimb during walking, after high pass filtering with Ithaco filters (data from NIH 15, level treadmill, 0.5 m/s)

B. Preliminary Histological Findings

Male cats were chronically implanted with recording cuffs for a period ranging from 180 to 300 days as described by Strange et al (1995a,b). Preliminary results show that this chronic implant schedule does not seem to cause damage that is quantifiable from histological samples. We interpret this to indicate that this long term implant protocol is safe for most animals. Further analysis is ongoing.

1. Methods

One centimeter long nerve samples were immersed in Karnovsky's fixative (Karnovsky, 1965), dehydrated and then osmicated for four hours. The osmicated samples were embedded in Jembed 812 (J.B.EM services, Quebec) and then 0.5 μm sections were cut using a glass knife. The 0.5 μm sections were counterstained with a 2:1 mixture of Richardson's stain and Toluidine blue. Sections were examined under a light microscope and photographs were taken at X800 magnifications using a dry objective. The photographs were developed into 4" by 6" prints and then these prints were scanned by a Hewlett Packard Scan Jet Plus. The scanned images were saved as PICT files and then these files were imported into NIH Image 1.52, an image analysis software package. Following image enhancement, the outside and inside perimeters of the axons were separately traced with a mouse. The area and perimeters of the axons and fibers were computed with NIH Image and this information was used to compute the true circular axon and fiber diameters as well as the myelin sheath thickness (Auer, 1994). The data were not corrected to account for shrinkage due to histological processing.

2. Preliminary Results

A preliminary examination of the data indicates several interesting features. The nerve samples from beneath the nerve cuffs tend to show both a characteristic increase in the amount of epineurial connective tissue and an increase in the amount of extraneural connective tissue that encapsulates each cuffed sample. These two connective tissue zones are distinctly demonstrated under low magnification (Fig. 6b). The corresponding control samples do not demonstrate this phenomenon (Fig. 6a). Qualitatively, the axons of a cuffed and a non-cuffed nerve are virtually indistinguishable . Axonal shapes and size distributions appear to be similar between the control (Fig. 7a) and the cuffed (Fig. 7b) samples.

The histograms showing the distribution of the fiber diameters show the bimodal population distribution typically seen in many other nerves samples. Other researchers (Friede and Beuche, 1985; Usson et al., 1987) have suggested that the occurrence of these peaks represent two separate normally distributed populations which are centered around two different modal averages. In this case, the data shows a large group of fibers centered around 11 μm and a smaller population centered around 5 μm. The plots of myelin thickness versus the axon diameter further (Figs. 8b and 9b) support the separation of the distribution into a large diameter and a small diameter population. Although a nearly linear relationship is demonstrated between axon size and myelin sheath thickness, a clear break appears in the distribution of the larger and the smaller fibers. The larger axons (>8 μm) from one subpopulation as do the axons that are smaller than 8 μm

A close examination of the histograms of NIH 13 and NIH 11 (Figs. 8a and 9a) reveals that the distributions of both populations are similar. This result is somewhat surprising given that NIH 11 experienced a decrease in the ulnar nerve compound action potential (CAP) to 40% of its initial value (at day 0 of the experiment) while NIH 13 maintained its signal at 92% of its original value (Strange et al., 1995a,b) . We expected that there would be difference in the fiber distribution between the two populations that might help to account for the difference in ENG amplitude but this result does not appear to have occured. Further work is required to assess whether a differential loss of fibers located near the edge of the nerve could be contributing to these results. Research conducted by Rydevik and Lundborg (1977) suggests that there may be reason to expect that the more outwardly located neurons are more susceptible to damage.

Figure 6a: Histology of NIH 13 distal Ulnar nerve control, low magnification (45X)

Figure 6b: Histology of NIH 13 distal Ulnar nerve experimental, low magnification (45X)

Figure 7a: Histology of NIH 14 distal radial nerve control. High magnification (800X)

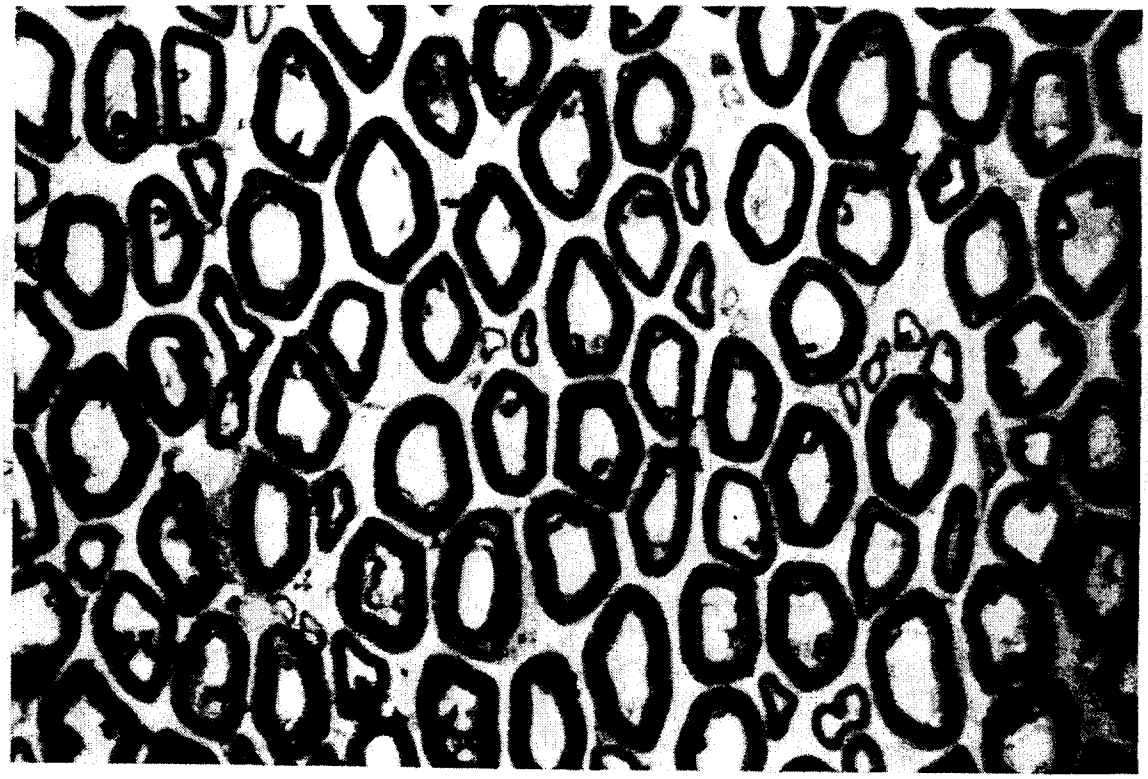

Figure 7b: Histology of NIH 14 distal radial nerve experimental. High magnification (800X)

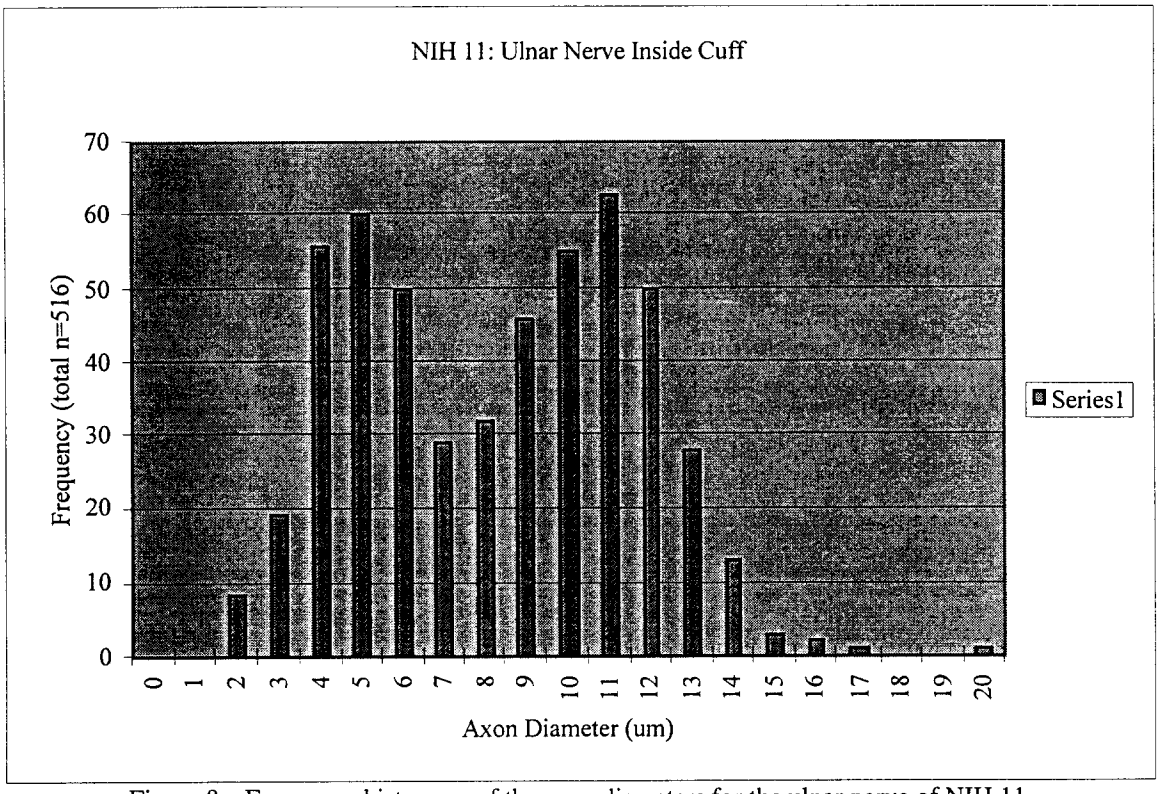

Figure 8a: Frequency histogram of the axon diameters for the ulnar nerve of NIH 11.

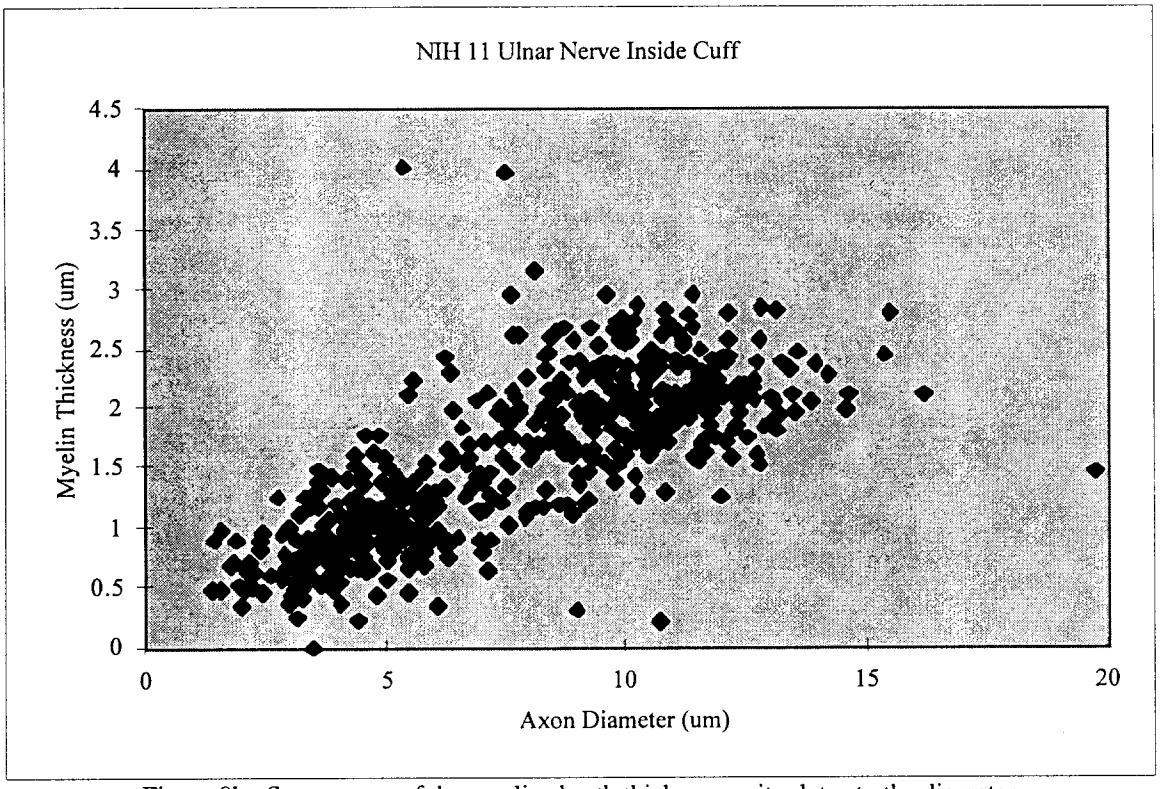

Figure 8b: Scattergram of the myelin sheath thickness as it relates to the diameter of the axon for the ulnar nerve of NIH 11..

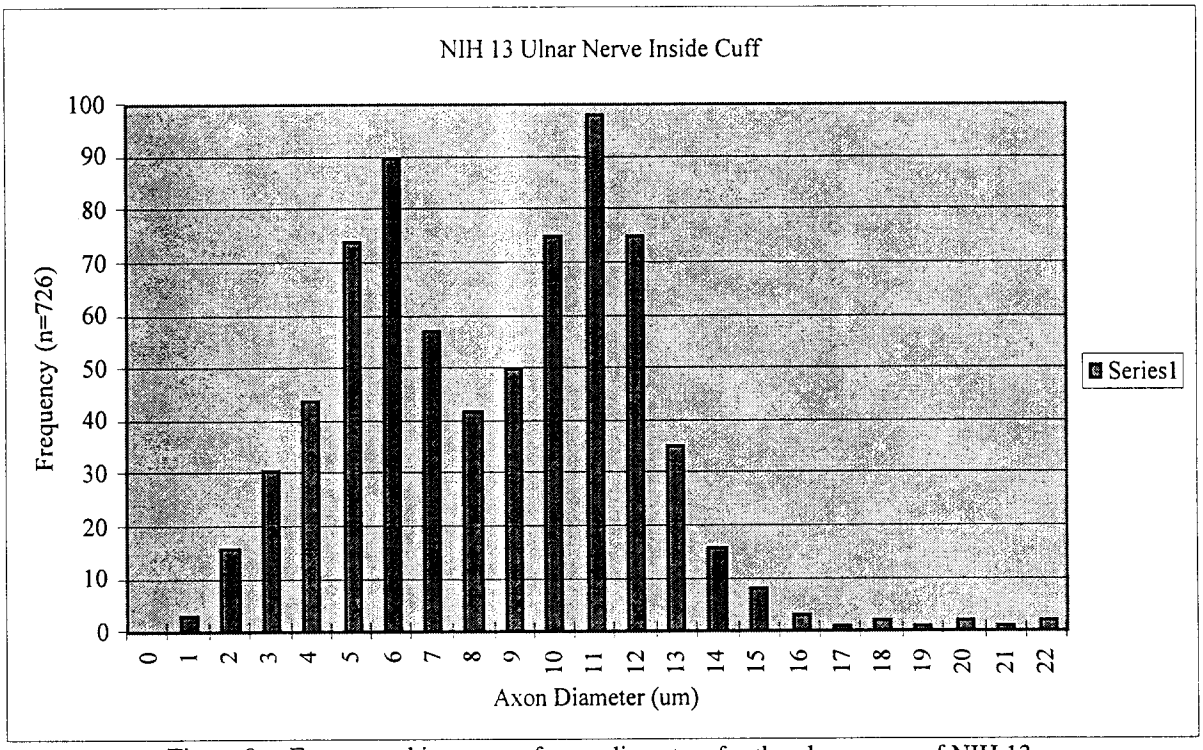

Figure 9a: Frequency histogram of axon diameters for the ulnar nerve of NIH 13.

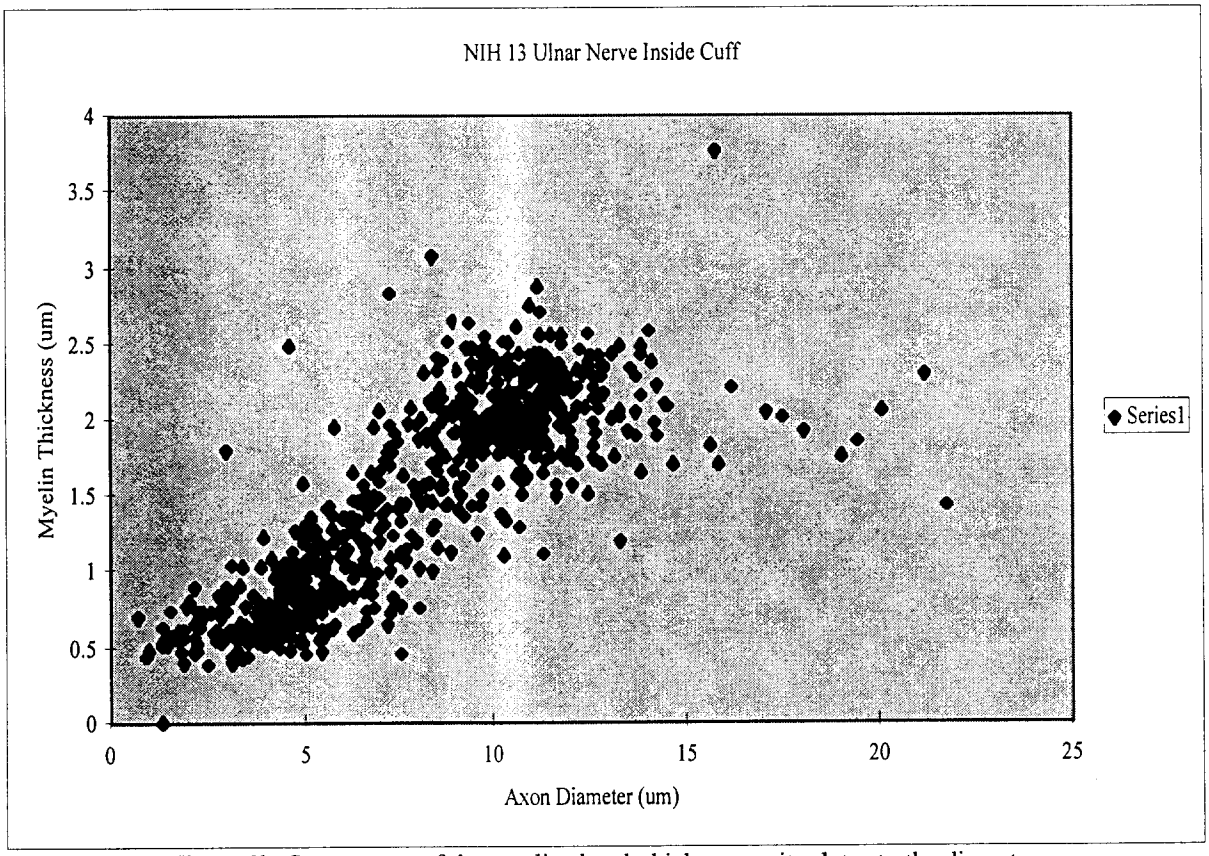

Figure 9b: Scattergram of the myelin sheath thickness as it relates to the diameter of the axon for the ulnar nerve of NIH 13.

C. Progress with Collaborators

During the eleventh quarter, we continued our collaboration with Drs. A. Kostov and R.B. Stein of the University of Alberta, concentrating on developing manuscripts based on our investigations of implementing machine learning techniques to analyze neural signals and predict muscle activity. We have developed a plan for further experiments to analyze data developed in the Year Two series of NIH implants as outlined in Progress Report #10 and future implementations of machine learning techniques in closed-loop control of functional electrical stimulation.

D. Publications and Meetings

1. Publications

During the eleventh quarter, the investigators developed three manuscripts for peer-reviewed journals based on results obtained from the current NIH contract. The three manuscripts are nearly ready for submission, and are outlined as follows:

Manuscript 1:

Title: Long term stability of nerve cuff signals recorded from the cat forelimb.

Authors: K. Strange, K. Kallesøe, and J.A. Hoffer

Manuscript 2:

Title: Sensory signals from the cat paw cutaneous receptors during walking: Applicability for closed-loop control of FES.

Authors: K. Strange and J.A. Hoffer

Manuscript 3:

Title: Artificial neural networks application in EMG-prediction using sensory nerve signals recorded in the cat's forelimb during walking.

Authors: A. Kostov, K. Strange, R.B. Stein, and J.A. Hoffer

2. Meetings

During the eleventh quarter, Kevin Strange and Andy Hoffer had an abstract published in the Proceedings of Neuroscience 25th Annual Meeting, Nov. 1995, San Diego, CA. The abstract is included in Appendix A.

IV. Plans for Twelfth Quarter

In the twelfth quarter we intend to:

1. examine histopathologically the nerves from Year One and Year Two cats (objective 5)
2. continue Year Three series of implants, implanting cuffs appropriate for smaller proprioceptive nerves (objective 3)
3. complete the construction of an 8-channel stimulator to be used for FES of forelimb muscles (objective 4b)
4. complete the construction of hardware and begin the software design for controlling the reaching task (objective 4a,b)
5. develop a model of closed loop control of FES during walking utilizing neural feedback (objective 4)
6. analyze walking data with our collaborators (objective 7)

V. References

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Strange K.D, Kallesoe, K., and Hoffer, J.A., (1995b), Long Term Stability of Nerve Signals Recorded in the Cat Forelimb, in preparation.

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VI. Appendix A

Abstract published in Soc. Neuroscience, Abstr. 21: 419, Nov. 1995, San Diego, CA.

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CUTANEOUS NEURAL FEEDBACK CAN BE USED TO PREDICT TIMING OF MUSCLE ACTIVATION IN THE CAT FORELIMB DURING LOCOMOTION.

K. D. Strange[2] and J. A. Hoffer[1,2*]. Schools of Kinesiology[1] & Engineering Science[2], Simon Fraser University, Burnaby, B.C., V5A 1S6, Canada

The objective of this research is to implement a closed-loop functional electrical stimulation (FES) system where sensory activity is used as feedback to control the timing and patterns of stimulation of paralyzed muscles. In this study, the electroneurograms (ENGs) recorded from cutaneous sensory nerves in awake cats walking on a treadmill were used to predict the timing of electromyogram (EMG) activity simultaneously recorded from wrist muscles.

Recording nerve cuffs were implanted in the left forelimb of six male cats around two of the Ulnar, Median, and/or Superficial Radial nerves, below the elbow where the motor functions of these nerves are minimal. Intramuscular EMG electrodes were implanted in Palmaris Longus (PalL) and three other wrist muscles. Nerve cuff and EMG signals obtained during walking were amplified, band-pass filtered and stored on magnetic tape. The limb movements were videotaped to correlate physiological signals with events and phases of the gait cycle.

ENG and EMG signals were sampled off-line and digitally rectified, bin-integrated to 10 ms bins, and normalized. A state-machine controller was designed which utilized Median and Radial cutaneous ENG signals as feedback inputs to predict the timing of onset and termination of PalL activity in the cat forelimb during locomotion. The controller specifically looked for features in the ENGs that were related to paw contact and lift-off. The resulting output signals were robust, and were unaffected by step duration and gait speed (0.5 - 1.0 m/s).

On the basis of these results we anticipate that clinical applications of FES controllers that include neural feedback should result in systems with increased accuracy and dependability.

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Key Words: (see instructions p. 4)

1. FES
2. Neural Prostheses
3. Closed-loop Control
4. Nerve Cuffs

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J. Hoffer	J. A. HOFFER	(604) 291 3141